The Important Herbal Pair for the Treatment of COVID-19 and Its Possible Mechanisms

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Research

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Abstract

Abstract Background: Coronavirus Disease 2019 (COVID-19) is an unprecedented disaster for people around the world. Many studies have shown that traditional Chinese medicine (TCM) are effective in treating COVID-19. However, it is difficult to find the most effective combination herbal pair among numerous herbs, as well as identifying its potential mechanisms. Herbal pair is the main form of a combination of TCM herbs, which is widely used for the treatment of diseases. It can also help us to better understand the compatibility of TCM prescriptions, thus improving the curative effects. The purpose of this article is to explore the compatibility of TCM prescriptions and identify the most important herbal pair for the treatment of COVID-19, and then analyze the active components and potential mechanisms of this herbal pair. M ethods: We first systematically sorted the TCM prescriptions recommended by the leading experts for treating COVID-19, and the specific herbs contained in these prescriptions across different stages of the disease. Next, the association rule approach was employed to examine the distribution and compatibility among these TCM prescriptions, and then identify the most important herbal pair. On this basis, we further investigated the active ingredients and potential targets in the selected herbal pair by a network pharmacology approach, and analyzed the potential mechanisms against COVID-19. R esult: We obtained 32 association rules for the herbal combinations in the selection of TCM treatment for COVID-19. The results showed that the combination of Amygdalus Communis Vas (ACV) and Ephedra sinica Stapf (ESS) had the highest confidence degree and lift value, as well as high support degree, which can be used in almost all the stages of COVID-19, so ACV and ESS (AE) were selected as the most important herbal pair. There were 26 active ingredients and 44 potential targets, which might be related to the herbal pair of AE against COVID-19. The main active ingredients of AE against COVID-19 were quercetin, kaempferol, luteolin, while the potential targets were Interleukin 6 (IL-6), Mitogen-activated Protein Kinase 1 (MAPK)1, MAPK8, Interleukin-1β (IL-1β), and Nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kB) p65 subunit (RELA). The protein-protein interaction (PPI) cluster demonstrated that IL-6 was the seed in the cluster, which plays an important role in connecting other nodes in the PPI network. The potential pathways mainly involved tumor necrosis factor (TNF), Toll-like receptor (TLR), hypoxia-inducible factor-1 (HIF-1), and nucleotide-binding oligomerization domain (NOD)-like receptor (NLRs). C onclusion: The combination of ACV and EAS was the most important herbal pair for the treatment of COVID-19. AE might have therapeutic effects against COVID-19 by affecting the inflammatory and immune responses, cell apoptosis, hypoxia damage and other pathological processes through multiple components, targets and pathways..

Background

Corona Virus Disease 2019 (COVID-19), that was discovered in December 2019, is an unprecedented disaster for people around the world. This disease caused by a novel coronavirus(SARS-CoV-2) has the characteristics of strong infectivity, rapid development and general susceptibilities[1]. The patients with COVID-19 showed typical respiratory symptom (such as fever, cough and lung damage) and some other

symptoms such as fatigue, myalgia, and diarrhea[2]. Many studies have found that even asymptomatic people also have strong infectivity[3, 4]. At present, specific antiviral drugs or vaccines currently have not been available for the treatment. main treatment strategy for COVID-19 is supportive care, which is supplemented by the combination of broad-spectrum antibiotics, antivirals, corticosteroids and convalescent plasma[5].

In China, it has become a consensus to combine traditional Chinese and Western medicine to improve the curative effect and reduce the mortality rate[6]. The facts have shown that traditional Chinese medicine (TCM) always contribute to effective treatment at critical moments, such as Severe Acute Respiratory Syndrome (SARS) in 2003 and influenza A (H1N1) in 2009[7]. There have been several studies finding that Integrated Medicine had better effects in improve the cure rate and overall response rate and did not increase adverse drug reactions for COVID-19[8]. Up to now, the seven version of “Diagnosis and treatment of novel coronavirus pneumonia” have been issued by the National Health Commission of the People’s Republic of China, which are mainly based on the characteristics of different stages of disease and the symptoms of patients[9]. Many studies have shown that traditional Chinese medicines, such as Lianhua Qingwen capsule, Qingfei Paidu decoction, are indeed effective in treating COVID-19 by inhibiting virus replication and invasion or inflammatory response [10, 11].

The key to obtain satisfactory curative effect lies in two points[12], one is accurate syndrome differentiation, the other is reasonable compatibility of traditional medicine. In the process of TCM, grasping the combination laws among different herbs are of great significance to improve the treatment effect. However, due to a number of TCM prescriptions involved in the treatment of COVID-19, it is a hard work to find the effective combination laws among numerous herbs, especially for inexperienced doctors. What is more, the exploration of the active ingredients, potential targets and action mechanism of combined Chinese medicine is crucial to the precise treatment of TCM for COVID-19 in the next futher.

So the aim of this study is to employ the association rule approach to examine the distribution and combination laws among traditional medicines recommended by leading experts for treating COVID-19, and then picks out the most important herbal pair. On this basis, we further investigate the active ingredients and potential targets in the selected herbal pair by a network pharmacology approach that is a systematic method proposed by Shao Li from Tsinghua University[13]. The contributions of this study are twofold. First, by mining the association rules, we obtain many interesting rules to investigate the hidden relationships among the numerous prescriptions of TCM and select the most important herbal pair. Second, with network pharmacology approach, we can further analyze the effective components, potential targets and mechanism in the most important herbal pair, so as to provide reference for further experimental research and TCM precision therapy research of COVID-19.

Methods

Compatibility law of Chinese medicine

According to the Chinese medicine prevention and treatment plan formulated by domestic first-line experts in the diagnosis and treatment protocol for COVID-19, the TCM prescriptions recommended in different disease stages, such as observation period, mild stage, middle stage, severe stage and recovery stage were sorted out systematically. On this basis, each herb in these prescriptions was classified and summarized to extract the most commonly used Chinese medicine in the treatment of COVID-19. Then, the data mining method of association rules was used to mine the combination rule of traditional Medicine and find the important herbal pairs for the treatment of COVID-19. Association rules can deal with data which contains a large number of variables and interpret their relationships if a proper support and confidence are given[14], which can be done by the Apriori algorithm provided from the ‘arules’ package of the R Software.

The Apriori algorithm can be simplified as follows: First, determine all of the frequent sets that are satisfied with a minimum support degree and minimum confidence degree. Then, generate strong association rules from these frequent item sets. In this study, we set the support degree to 0.2 and the confidence degree to 0.6. The data format was sorted into "shopping basket" format. The herbs included in each prescription were analyzed to observe the "co-occurrence" of different traditional medicine, and the frequent items of data and the combination rules of herbal pairs were analyzed. Then, the most important drug pairs for COVID-19 were extracted for further network pharmacologic analysis.

Active ingredients and targets of the selected herbal pair

The ingredients of the selected herbal pair were obtained from the Traditional Chinese Medicine Systems Pharmacology Database (TCMSP, http://tcmspw.com/tcmsp.php/) and PubChem Database (https://pubchem.ncbi.nlm.nih.gov/). TCMSP is a systematic pharmacology platform designed for herbs, which is capable of describing the relationship between drugs, targets and diseases[15]. PubChem is the world’s largest collection of accessible chemical information, which can provide the molecular formula, structure, biological activities and toxicity information of chemical compounds. In addition, the TCMSP database was used to identify potential targets of the herbal pair, and their gene names were obtained from the UniProt database (https://www.uniprot.org/) by limiting the species with “Homo sapiens”.

In this section, the active ingredients were extracted based on pharmacokinetic evaluation (absorption, distribution, metabolism, excretion (ADME) properties of compounds) to extract the chemical ingredients with favorable pharmacokinetics properties[16]. Therefore, we employed two important ADME-related properties, namely, oral bio-availability (OB) and drug-likeness (DL), in our study to explore the potential bio-active compounds of AE. The ingredients with OB $\geq$ 30% and DL $\geq$ 0.18 were selected in this study, and all of the candidate compounds were approved by literature.

Potential targets of the herbal pair for COVID-19

The data for the COVID-19-associated target genes were obtained from Genecards database (https://www.genecards.org/) and the Online Mendelian Inheritance in Man (OMIM) database(https://omim.org/). Genecards is an extensive platform which provides insight into predicted and annotated human genes. All of the gene-centric data were gathered from 150 web resources, including genetic, genomic, transcriptomic, proteomic and functional information[17]. The OMIM database links and catalogues all known diseases with a genetic component and provides further references to genomic analyses of catalogued genes[18] .The keyword was set as “novel coronavirus pneumonia”.

The mapping of COVID-19 disease targets and drug targets was carried out by the R (3.6.2) software to identify the intersection targets as potential targets. Then, we obtained the protein–protein interaction(PPI) data of intersection targets from the STRING database (https://string-db.org/). By choosing the “multiple proteins” mode and setting the protein species as “homo sapiens”. The STRING database defines PPI with confidence ranges for data scores (high > 0.7; medium > 0.4; low > 0.15)[19]. In this study, we selected a confidence score of > 0.7 to construct our PPI network. Next, we imported the .tsv file into Cytoscape for further analysis.

Construction of “herb-component-target-disease” network

Network analysis was carried out to facilitate scientific interpretation of the complicated relationships among herbs, compounds, diseases and genes[20]. In this study, we generated the networks using Cytoscape (version 3.7.2). Firstly, the active components that are corresponding to the targets of COVID-19 were identified by R software, and the disease-related active components and potential targets were input into the Cytospace software to further construct the “herb-component-target-disease” network. The centrality of the network nodes was analyzed by CytoNCA. The degree value was used as the screening condition. The higher the degree value, the more targets the component was related to. In this way, we could analyze the disease-related core components in this herbal pair.

Enrichment Analysis

In this study, the background database “org. HS. Eg.db” of R3.6.2 was used to obtain the gene ID (Entrez ID) of the potential targets, and then the “clusterprofiler” package was used to analyze the Gene Ontology (GO) function enrichment of these potential targets, including three aspects: biological process (BP), cellular component (CC) and molecular function (MF). The P-value was set with a cutoff = 0.05 and q-value cutoff = 0.05, and each category was ranked by significance, and the top 10 enrichment items were displayed in form of a histogram. The Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway information of potential targets was obtained by the DAVID database. R software was used to analyze the gene number, significance, enrichment fold of target-related pathways by drawing a bubble diagram. Then, we import KEGG data into Cytoscape and conducted network analysis of the top 20 enrichment pathways with significant differences and their associated potential targets.

Results

Compatibility law of Chinese medicine for COVID-19

We collected a total of 24 TCM prescriptions including 105 herbs that are highlighted in the guidelines for COVID-19. According to the characteristics of the disease development, we summarized the Chinese medicine prescriptions available at different stages and its specific medicinal herbs in Fig. 1. It is found that different TCM prescriptions are recommended at different disease stages of COVID-19. Even at the same stage, the recommended TCM prescription varies according to the patient's specific syndromes. Among them, Qingfei Paidu Decoction is the most widely recommended, which can be uesed for mild, moderate and severe patients. Besides, Xuebijing Injection, Reduning Injection, Tanreqing Injection and Xingnaojing Injection were recommended twice, in severe and critical conditions. Besides, It can be found that almost every prescription consists of a variety of different herbs, and the same herb may appear in different prescriptions by a certain combination law.

To further explore the prescription rules of TCM treatment for COVID-19, we analyzed 105 kinds of herbs in the recommended TCM prescriptions and found that the most commonly used herbs includ Licorice, Gypsum, Ephedra sinica Stapf, Agastache rugosa, Amygdalus Communis Vas, Baikal Skullcap, Forsythia suspensa and so on. The first 20 commonly used Chinese herbs are shown in Fig. 2(A). Next, to generate association rules among these herbs, we performed data analysis based on association rules algorithm, with 24 sets of recommended TCM prescriptions in this study. On the premise of support of 0.2 and confidence of 0.6, we obtained 32 association rules for herb combinations in the process of TCM treatment for COVID-19, which can be seen in Table 1. In general, a big value for the lift ($X \Rightarrow Y$) indicates a stronger association between X and Y[21]. The lift values of these 32 rules are all greater than 1, which indicate positive interdependence effects in these rules.

Table 1
Meaningful association rules among traditional herbs for treating COVID-19

Rules	Confidence	Support	Lift
{Amygdalus Communis Vas} => {Ephedra sinica Stapf}	1.000	0.250	3.429
{Poria cocos} => {Agastache rugosa}	1.000	0.208	3.429
{Amygdalus Communis Vas} => {Gypsum}	1.000	0.250	3.000
{Ephedra sinica Stapf,Amygdalus Communis Vas} => {Gypsum}	1.000	0.250	3.000
{Gypsum,Amygdalus Communis Vas} => {Ephedra sinica Stapf}	1.000	0.250	3.429
{Ephedra sinica Stapf,Gypsum} => {Amygdalus Communis Vas}	1.000	0.250	4.000
{Licorice,Amygdalus Communis Vas} => {Ephedra sinica Stapf}	1.000	0.208	3.429
{Licorice,Ephedra sinica Stapf} => {Amygdalus Communis Vas}	1.000	0.208	4.000
{Licorice,Amygdalus Communis Vas} => {Gypsum}	1.000	0.208	3.000
{Licorice,Ephedra sinica Stapf} => {Gypsum}	1.000	0.208	3.000
{Licorice,Ephedra sinica Stapf,Amygdalus Communis Vas} => {Gypsum}	1.000	0.208	3.000
{Licorice,Gypsum,Amygdalus Communis Vas} => {Ephedra sinica Stapf}	1.000	0.208	3.429
{Licorice,Ephedra sinica Stapf,Gypsum} => {Amygdalus Communis Vas}	1.000	0.208	4.000
{Gypsum} => {Licorice}	0.875	0.292	1.909
{Ephedra sinica Stapf} => {Amygdalus Communis Vas}	0.857	0.250	3.429
{Ephedra sinica Stapf} => {Gypsum}	0.857	0.250	2.571
{Forsythia suspensa} => {Licorice}	0.833	0.208	1.818
{Amygdalus Communis Vas} => {Licorice}	0.833	0.208	1.818
{Ephedra sinica Stapf,Amygdalus Communis Vas} => {Licorice}	0.833	0.208	1.818
{Gypsum,Amygdalus Communis Vas} => {Licorice}	0.833	0.208	1.818
{Ephedra sinica Stapf,Gypsum} => {Licorice}	0.833	0.208	1.818
{Ephedra sinica Stapf,Gypsum,Amygdalus Communis Vas} => {Licorice}	0.833	0.208	1.818
{Gypsum} => {Amygdalus Communis Vas}	0.750	0.250	3.000
{Gypsum} => {Ephedra sinica Stapf}	0.750	0.250	2.571
{Agastache rugosa} => {Poria cocos}	0.714	0.208	3.429

Rules	Confidence	Support	Lift
{Agastache rugosa} => {Ephedra sinica Stapf}	0.714	0.208	2.449
{Ephedra sinica Stapf} => {Agastache rugosa}	0.714	0.208	2.449
{Agastache rugosa} => {Licorice}	0.714	0.208	1.558
{Ephedra sinica Stapf} => {Licorice}	0.714	0.208	1.558
{Licorice,Gypsum} => {Amygdalus Communis Vas}	0.714	0.208	2.857
{Licorice,Gypsum} => {Ephedra sinica Stapf}	0.714	0.208	2.449
{Licorice} => {Gypsum}	0.636	0.292	1.909

Screening of important herbal pairs

We futher extracted the combination itmes containing two herbs by subset function and make a graph for it. There were 16 rules for the combination law of herbal pairs. According to the confidence ranking, {Amygdalus Communis Vas}=> {Ephedra sinica Stapf}, {Poria cocos}=>{Agastache rugosa},{Amygdalus Communis Vas}=>{Gypsum},{Gypsum} => {Licorice}, {Ephedra sinica Stapf} =>{Amygdalus Communis Vas} and {Ephedra sinica Stapf}=>{Gypsum} were the top six important herbal pairs, which can be seen in the Fig. 2(B). The larger the circle between the two herbs, the higher the confidence level, and the darker the color, the higher the lift value. Among them, {Amygdalus Communis Vas}=>{Ephedra sinica Stapf} has the highest confidence degree(1.00) and lift value(3.43) as well as high support degree(0.25). This means that the probability of using both herbs at the same time is 25%. Under the premise of using Amygdalus Communis Vas, the probability of using Ephedra sinica Stapf is 100%, which is 3.43 times higher than that of using Ephedra sinica Stapf alone. Additionally, further analysis found that the herbal pair of Amygdalus Communis Vas(ACV) and Ephedra sinica Stapf (ESS) is widely used in almost all stages of disease, including observation period, mild cases, general cased, severe and critical cases. So the combination of Amygdalus Communis Vas and Ephedra sinica Stapf was picked as the most important herbal pair in the process of treating COVID-19.

Active ingredients of AE

Based on the above analysis, the combination of ACV and ESS (AE) was regarded as the most important herbal pair and its' active ingredients and potential targets were futher studied. 40 active ingredients from 476 compounds of AE met the requirements of OB $\geq$ 30% and DL $\geq$ 0.18. It was found that 5 active components had no corresponding action targets, and the other 35 active ingredients that can also be searched by literatures had a total of 210 targets(Table 2). Among these 35 active ingredients, 21 ingredients are from ESS, while 16 ingredients are from ACV, and 2 ingredients ((+)-catechin, stigmasterol)

belong to both herbs. The 3D structures of the top five ingredients of ESS and ACV with the largest number of targets were shown in Fig. 3.

Table 2
Active ingredients of ACV and ESS.

Herb	Mol Name	Mol Name	OB%	DL	Targets
ESS	MOL000098	quercetin	46.43	0.28	154
	MOL000422	kaempferol	41.88	0.24	63
	MOL000006	luteolin	36.16	0.25	57
	MOL000358	beta-sitosterol	36.91	0.75	38
	MOL004328	naringenin	59.29	0.21	37
	MOL000449	Stigmasterol	43.83	0.76	31
	MOL002823	Herbacetin	36.07	0.27	14
	MOL005573	Genkwanin	37.13	0.24	14
	MOL004576	taxifolin	57.84	0.27	12
	MOL005842	Pectolinarigenin	41.17	0.3	12
	MOL000492	(+)-catechin	54.83	0.24	11
	MOL002881	Diosmetin	31.14	0.27	10
	MOL005190	eriodictyol	71.79	0.24	9
	MOL011319	Truflex OBP	43.74	0.3	7
	MOL010788	leucopelargonidin	57.97	0.24	5
	MOL010489	Resivit	30.84	0.27	4
	MOL007214	(+)-Leucocyanidin	37.61	0.27	4
	MOL001494	Mandenol	42	0.19	3
	MOL001755	24-Ethylcholest-4-en-3-one	36.08	0.76	2
	MOL001771	poriferast-5-en-3beta-ol	36.91	0.75	2
	MOL005043	campest-5-en-3beta-ol	37.58	0.71	1
ACV	MOL000449	Stigmasterol	43.83	0.76	31
	MOL012922	l-SPD	87.35	0.54	30
	MOL010921	estrone	53.56	0.32	25
	MOL004908	Glabridin	53.25	0.47	25
	MOL007207	Machiline	79.64	0.24	21
	MOL004841	Licochalcone B	76.76	0.19	19

Herb	Mol Name	Mol Name	OB%	DL	Targets
	MOL005017	Phaseol	78.77	0.58	14
	MOL000492	(+)-catechin	54.83	0.24	11
	MOL002311	Glycyrol	90.78	0.67	11
	MOL004903	liquiritin	65.69	0.74	6
	MOL000953	CLR	37.87	0.68	4
	MOL000359	sitosterol	36.91	0.75	3
	MOL004355	Spinasterol	42.98	0.76	3
	MOL005030	gondoic acid	30.7	0.2	2
	MOL002211	11,14-eicosadienoic acid	39.99	0.2	1
	MOL000211	Mairin	55.38	0.78	1

“AE-component-target-COVID” network

A total of 261 COVID-19 disease targets were obtained from GeneCards and OMIM database, And 44 crossed targets of AE and COVID-19 were obtained by R software, as shown in Fig. 4A. These crossed targets were regarded as the potential targets of AE against COVID-19. Then, the “AE-component-target-disease” network was constructed by Cytoscape, as depicted in Fig. 4C. This network includes 73 nodes and 225 edges, with a network density of 0.081 and a network diameter of 4. The key nodes in this network were showed in Table 3. The topological parameters showed that the node-degree distribution obeyed the power-law distribution (Fig. 4B). There were 43 ESS targets, 7 ACV targets and 6 overlapped targets (PTGS2, PTGS1, CAT, NOS2, PPARG, and SOD1), which involved a total of 26 active ingredients of AE. According to the degree value, the most critical ingredients of AE are quercetin, luteolin, kaempferol, naringenin and (+)-catechin, which interact with 38, 18, 13, 10 and 7 targets of COVID-19, respectively.

Table 3
Key nodes of “AE-component-target-COVID”network and their topological characteristics

Component	Betweenness centrality	Degree	Targets	Betweenness centrality	Degree
quercetin	0.258	39	PTGS2	0.209	29
luteolin	0.068	19	PTGS1	0.147	25
kaempferol	0.032	14	PPARG	0.036	13
naringenin	0.025	11	NOS2	0.019	9
beta-sitosterol	0.013	8	RELA	0.007	6
(+)-catechin	0.007	8	CASP3	0.006	6
Stigmasterol	0.003	6	SOD1	0.009	5
Licochalcone B	0.008	6	MAPK14	0.010	5
taxifolin	0.004	5	ICAM1	0.005	5
Glycyrol	0.005	5	HMOX1	0.006	5
Glabridin	0.005	5	CAT	0.003	5

PPI network of AE against COVID-19

PPI networks have been widely used to identify many different interactions of protein targets in the context of complex disease. There were a total of 44 nodes and 324 interaction lines in the STRING PPI network. The PPI enrichment P-value was less than 1.0e-16, demonstrating an obvious protein interaction relationship, which was shown in Fig. 5(A). Due to the complexity of the original network obtained from the STRING database, we imported the PPI data into Cytoscape to analyze the importance of targets in protein networks and the main cluster in this network. The node represents the potential targets, and the larger the node area and the redder the color, the more important the target protein is. As shown in Fig. 5(B), it indicates that interleukin 6 (IL-6), mitogen-activated protein kinase (MAPK) 1, MAPK8, interleukin-1β (IL-1β), nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kB) p65 subunit (RELA), C-X-C motif chemokine ligand 8 (CXCL-8), C-C motif chemokine ligand 2 (CCL2) and prostaglandin G/H synthase 2 (PTGS2) were the key target proteins for the treatment of COVID-19 with AE. Among them, IL-6 (degree = 32) is the most critical target in the PPI network, and its main cluster network was shown in Fig. 3C.

Enrichment of potential targets of AE

To further explore the mechanisms of AE as a therapy against COVID-19, we performed GO enrichment and KEGG analysis with the 44 potential targets identified by R software. GO enrichment consists of three

parts: biological process (BP), cellular component (CC) and Molecular function (MF). There were 1633 enrichment terms of GO for BP, including response to lipopolysaccharide (LPS), bacterial molecules, biotic stimulus and oxidative stress, positive regulation of cytokine production and so on. Besides, a total of 32 CC items were obtained through the enrichment analysis of GO, including membrane raft, microdomain and region, caveola, plasma membrane raft, outer membrane, focal adhesion and so on. There were 78 terms of GO for MF enrichment, such as cytokine receptor binding and activity, receptor ligand activity, chemokine receptor binding, phosphatase binding, MAP kinase activity, and chemokine activity. The top 10 most important GO analysis items for different categories are shown in Fig. 6. In KEGG analysis, we obtained a total of 113 pathways, which were from several categories, such as human diseases, signal transduction, cell process and immune system. The top 20 significantly enriched KEGG pathways were presented in Fig. 7(A). The main pathways were tumor necrosis factor (TNF), Toll-like receptor (TLR), hypoxia-inducible factor-1 (HIF-1), nucleotide-binding oligomerization domain (NOD)-like receptor(NLRs) and several disease-related pathways, such as Chagas disease and Influenza A, which were regarded as the important pathways related to AE against COVID-19. The network of the top 20 pathways and their targets displayed in Fig. 7(B), where the size of the label font represents the degree value of the node in the network. The detailed information of the gene targets in these 20 pathways was listed in Table 4.

Table 4
Targets distribution information of the top 20 enriched KEGG pathways.

Term	Discription	Genes
hsa05152	Tuberculosis	IL6, RELA, STAT1, IL10, MAPK1, CASP3, BAX, MAPK14, BCL2, CASP8, IFNG, MAPK3, IL1B, MAPK8, NOS2, IL1A
hsa05145	Toxoplasmosis	RELA, BCL2L1, STAT1, IL10, MAPK1, CASP3, CD40LG, MAPK14, BCL2, CASP8, IFNG, MAPK3, MAPK8, NOS2
hsa04620	Toll-like receptor signaling pathway	IL6, RELA, CXCL8, STAT1, CXCL11, CXCL10, MAPK1, FOS, MAPK14, CASP8, MAPK3, IL1B, MAPK8
hsa04668	TNF signaling pathway	ICAM1, IL6, CCL2, PTGS2, RELA, CXCL2, CXCL10, MAPK1, FOS, CASP3, MAPK14, CASP8, MAPK3, IL1B, MAPK8
hsa04660	T cell receptor signaling pathway	IL4, MAPK1, FOS, CD40LG, MAPK14, RELA, MAPK3, IFNG, IL10, IL2
hsa05132	Salmonella infection	IL6, RELA, CXCL2, CXCL8, MAPK1, FOS, MAPK14, IFNG, MAPK3, IL1B, MAPK8, NOS2, IL1A
hsa05133	Pertussis	IL6, RELA, CXCL8, IL10, MAPK1, FOS, CASP3, MAPK14, MAPK3, IRF1, IL1B, MAPK8, NOS2, IL1A
hsa05200	Pathways in cancer	PRKCA, EGFR, IL6, PTGS2, RELA, PPARG, CXCL8, RB1, BCL2L1, STAT1, PRKCB, MAPK1, FOS, CASP3, BAX, BCL2, CASP8, MAPK3, MAPK8, NOS2
hsa04380	Osteoclast differentiation	MAPK1, FOS, MAPK14, RELA, MAPK3, PPARG, IFNG, IL1B, MAPK8, STAT1, IL1A
hsa04621	NOD-like receptor signaling pathway	MAPK1, IL6, CCL2, MAPK14, RELA, MAPK3, CASP8, CXCL2, CXCL8, IL1B, MAPK8
hsa04010	MAPK signaling pathway	PRKCA, EGFR, FOS, MAPK1, CASP3, MAPK14, RELA, MAPK3, HSPB1, IL1B,
hsa05144	Malaria	ICAM1, IL6, CCL2, CD40LG, IFNG, CXCL8, IL1B, IL10
hsa05140	Leishmaniasis	IL4, PTGS2, RELA, STAT1, IL10, FOS, MAPK1, MAPK14, IFNG, MAPK3, IL1B, NOS2, IL1A
hsa05164	Influenza A	PRKCA, ICAM1, IL6, CCL2, RELA, CXCL8, STAT1, PRKCB, CXCL10, MAPK1, MAPK14, IFNG, MAPK3, IL1B, MAPK8, IL1A
hsa05321	Inflammatory bowel disease (IBD)	IL4, IL6, RELA, IFNG, IL1B, STAT1, IL10, IL1A, IL2
hsa04066	HIF-1 signaling pathway	PRKCA, EGFR, MAPK1, IL6, RELA, HMOX1, BCL2, MAPK3, SERPINE1, IFNG, NOS3, NOS2, PRKCB

Term	Discription	Genes
hsa05161	Hepatitis B	PRKCA, FOS, MAPK1, CASP3, IL6, BCL2, RELA, BAX, CASP8, MAPK3, CXCL8, MAPK8, RB1, STAT1, PRKCB
hsa04060	Cytokine-cytokine receptor interaction	IL4, IL6, CCL2, CXCL2, CXCL8, CXCL11, IL10, CXCL10, CD40LG, IFNG, IL1B, IL1A, IL2
hsa05142	Chagas disease (American trypanosomiasis)	IL6, CCL2, RELA, CXCL8, IL10, MAPK1, FOS, MAPK14, CASP8, IFNG, SERPINE1, MAPK3, IL1B, MAPK8, NOS2, IL2
hsa05146	Amoebiasis	PRKCA, CASP3, IL6, RELA, IFNG, CXCL8, HSPB1, IL1B, NOS2, IL10, PRKCB

Discussion

Until now, people around the world are still actively fighting COVID-19. Traditional medicine has played an important role in this epidemic, especially in China. Recent reports from the Information Office of the State Council of China showed that 74,187 people with COVID-19 received TCM treatment, accounting for 91.5% of all cases in China. Even in Hubei province, there were 61,449 patients (90.6%) treated by TCM. It has been shown that the total effective rate of TCM treatment for COVID-19 was over 90%. From the above association rule analysis, Amygdalus Communis Vas and *Ephedra sinica Stapf* is one of the most commonly used herbal pair for COVID-19, which was not only included in many TCM prescriptions recommended by experts across the country, but also used almost throughout the course of COVID-19. According to the theories of TCM, the main cause of the disease is “dampness toxin”, and it is mainly affecting the lung and spleen[22]. Dampness has the characteristics of heavy turbid and sticky, which can hinder the movement of Qi and blood, thereby causing lung and spleen dysfunction. ESS has the effects of inducing perspiration, dispelling dampness, diffusing the Lung Qi and relieving cough and asthma, while ACV has the effects of depressing Qi and suppressing cough and anti-asthma. The combination of ESS and ACV can exert the efficacy of normalizing Qi dynamic and relieving cough and asthma. However, its potential mechanisms against COVID-19 have not been fully explored yet. So we applied the network pharmacology approach to futher explore the active compounds and potential targets and mechanisms of AE against COVID-19.

In this study, there were 26 active ingredients and 44 potential targets, which may be relate to AE against COVID-19. After calculation, we find that each herb was interacted with an average of 74.5 targets, each compound was interacted with an average of 5.73 targets, and each target was interacted with an average of 3.48 compounds. These results also indicate the fact that traditional medicines, like ESS and ACV, have the characteristics of multiple compositions and interaction targets. The main active ingredients of AE against COVID-19 are quercetin, kaempferol, luteolin. According to previous studies, quercetin has multiple biological activities, such as anti-inflammatory, anti-viral, anti-oxidative, anti-tumor actions[23, 24]. A study reported that quercetin could interact with the HA2 subunit of Influenza A virus

(IAVs), and it could inhibit the entry of the H5N1 virus using the pseudovirus-based drug screening system[25]. Bacterial LPS activates the translocation of NF-κB by binding to the TLR on the surface of the cell membrane, thereby triggering the inflammatory responses, while quercetin can block the activation of TLR and inhibit the expression of LPS-induced adhesion molecules and inflammatory factors on the surface of the membrane[26]. Additionally, kaempferol has anti-inflammatory, anti-oxidative, anti-bacterial, anti-viral and other effects[27], including the inhibition of the replication of bovine herpes virus and LPS-induced inflammatory responses[28]. It could also alleviate acute lung injury induced by H9N2 swine influenza virus by inhibiting TLR4/myeloid differentiation factor 88 (MyD88)-mediated NF-κB and MAPK signaling pathways[29]. On the other hand, luteolin has anti-inflammatory, anti-allergic, anti-bacterial, anti-viral and other effects[30]. For example, luteolin inhibits the replication of dengue virus by inhibiting pro-protein-converting enzyme activity[31], and it also has potent anti-viral activity against Japanese encephalitis virus replication in non-small-cell lung carcinoma A549 cells[32]. In short, all these suggested that the active components of AE can be used to treat many diseases with multi-target regulation, especially for viral infection diseases.

According to the literature, angiotensin-converting enzyme2 (ACE2) and 3C-like protease (3CL pro) are regarded as the critical targets for antiviral drug design[33, 34]. Traditional medicine could target ACE2 to prevent SARS-CoV-2 from entering into host cells or target 3CL pro to inhibit the replication and assembly of the virus in cells. For example, Li finded that the Lianhua Qinwen capsule significantly inhibited the replication of SARS-CoV-2 with an IC50 value of 411.2 μg/mL in Vero E6 cells[10]. Fan reported that the active ingredients in Qingfei Paidu decoction might act directly on the SARS-CoV-2 3CL pro to block virus proliferation[35]. Besides, the virus can induce cell damage and induce a series of immune and inflammatory responses after entering into the cells. Especially in severe and critical patients, cytokine storms are closely related to the development of the disease. In this study, 44 potential targets were obtained through the intersection of the disease targets of COVID-19 and the action targets of AE, and these targets are mainly related to inflammatory or immune factors, such as IL-6, MAPK1, MAPK8, IL1B, RELA, CXCL-8, CCL2, PTGS2. Furthermore, ESS and ACV might have synergistic effects, as they have common disease targets and complementary disease targets. From the PPI network, IL-6 was found to be the most critical target. IL-6 is a powerful inducer of acute-phase reaction, which can act on B and T cells, liver cells, hematopoietic progenitor cells and central nervous system cells, and can induce multiple biological processes[36]. A recent study also shows that after SARS-COV-2 infection, $CD4^+$ T cells were rapidly activated, and then proliferated and differentiated into Th1 cells that can produce granulocyte-macrophage colony stimulating factor (GM-CSF). GM-CSF further induced inflammatory CD14 + CD16 + monocytes to express IL-6 and other factors to accelerate inflammation. So it is speculated that GM-CSF and IL-6 may be the key to induce cytokine storm[37]. Additionaly, GO enrichment analysis revealed that the biological processes of AE against COVID-19 involved cytokine activity, anti-oxidative stress response, cell apoptosis, and cellular response to LPS and bacteria-derived molecules. The molecular functions of AE mainly included cytokine receptor binding, cytokine and receptor ligand activities, chemokine receptor binding, phosphatase binding, MAP kinase activity, and chemokine activity. From the KEGG enrichment,

the results indicates that the key signaling pathways of AE aganist COVID-19 were TNF, TLR, HIF-1 and NLRs signaling pathways that closely related to inflammation, immunity and oxidation process. Many studies of TCM against COVID-19 also reported that the anti-COVID-19 mechanism of TCM prescriptions including AE might involve the inflammation and immunity pathways liking TLR, JAK-STAT, TNF, AGE-RAGE, T cell receptor, IL-17 and so on[38, 39].

In summary, this study first employed the association rule approach to examine the distribution and combination laws among traditional medicines recommended by leading experts for treating COVID-19, which allows us to better grasp the clinical use of Traditional medicine treatment. Then, we picked out the most important herbal pair– *Amygdalus Communis Vas* and *Ephedra sinica Stapf*. On this basis, we further investigate the active ingredients and potential targets in the selected herbal pair by a network pharmacology approach. The results suggested that AE has therapeutic effects against COVID-19 by affecting the pathological processes such as inflammatory and immune responses, cell apoptosis, hypoxia damage and other pathological processes through multiple components, multiple targets and multiple pathways. The above predicted TCM targets and pathways can further guide the next research. However, it should be noted that, there is still lack of cognition on the pathogenesis of COVID-19 at this stage. In addition, different methods of computational chemistry and biology have differences in results and limitations, so it could be possible that the calculation results are not consistent with the actual application effects. Therefore, it is necessary to further conduct *in vitro* and *in vivo* experiments to confirm the efficacy of ESS and ACV combination for the treatment of COVID-19, thus providing more information for its development and clinical application.

Conclusion

Amygdalus Communis Vas and Ephedra sinica Stapf was the most important herbal pair in the treatment of COVID-19. The main active ingredients of AE against COVID-19 were quercetin, kaempferol, luteolin and the important targets were IL-6,MAPK1, MAPK8, IL-1β, and RELA. AE may have therapeutic effects against COVID-19 by affecting the pathological processes such as inflammatory and immune responses, cell apoptosis, hypoxia damage and other pathological processes through multiple components, multiple targets and multiple pathways.

Abbreviations

ACE2: Angiotensin-converting enzyme2; ACV: Amygdalus Communis Vas; ADME: Absorption, distribution, metabolism, excretion; BP:Biological process; CC:Cellular component; CCL: C-C motif chemokine ligand; COVID-19: Corona virus disease 2019; CXCL: C-X-C motif chemokine ligand; DL: Drug-likeness; ESS: Ephedra sinica Stapf; GM-CSF: Granulocyte-macrophage colony stimulating factor; GO: Gene Ontology; H1N1: Influenza A; HIF-1: hypoxia-inducible factor-1; IAVs: Influenza A virus; IL-6: Interleukin 6; KEGG: Kyoto Encyclopedia of Genes and Genomes; LPS: Lipopolysaccharide; MAPK: Mitogen-activated protein kinase; MF: Molecular function; NF-kB: Nuclear factor kappa-light-chain-enhancer of activated B cells; NOD: Nucleotide-binding oligomerization domain; OB: Oral bio-availability; OMIM: Online mendelian

inheritance in man; PPI: Protein-protein interaction; PTGS: Prostaglandin G/H synthase; RELA: Nuclear factor kappa-light-chain-enhancer of activated B cells p65 subunit; SARS: Severe acute respiratory syndrome; TCM: Traditional Chinses medicine; TCMSP: Traditional chinese medicine systems pharmacology database; TLR: Toll-like receptor; TNF: Tumor necrosis factor.

Declarations

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Authors’contributions

Conceptualization, S.X and C.L; Methodology, S.X, J.C and B.G.; Software, S.X; Validation, S. X., Z. Z., B. G., C.T.V., J.C., S. C., X.L., G.C.and C. L. ; Formal Analysis, S.C. and X.L.; Investigation, J.C. and S.C.; Resources, C.L.; Data Curation, C.T.V. and Z.Z.; Writing-Original Draft Preparation, S.X.; Writing-Review & Editing, Z.Z. and C.T.V. ; Visualization, S.X. and B.G.; Supervision, G.C.and C.L.; Project Administration, C.L; Funding Acquisition, C.L.

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Availability of data and materials

The data used to support the findings of this study are available from the corresponding author upon request.

Ethics approval and consent to participate

Not applicable.

Consent for publication

The manuscript is approved by all authors for publication.

Competing interests

The authors report no conflicts of interest in this work.

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Figures

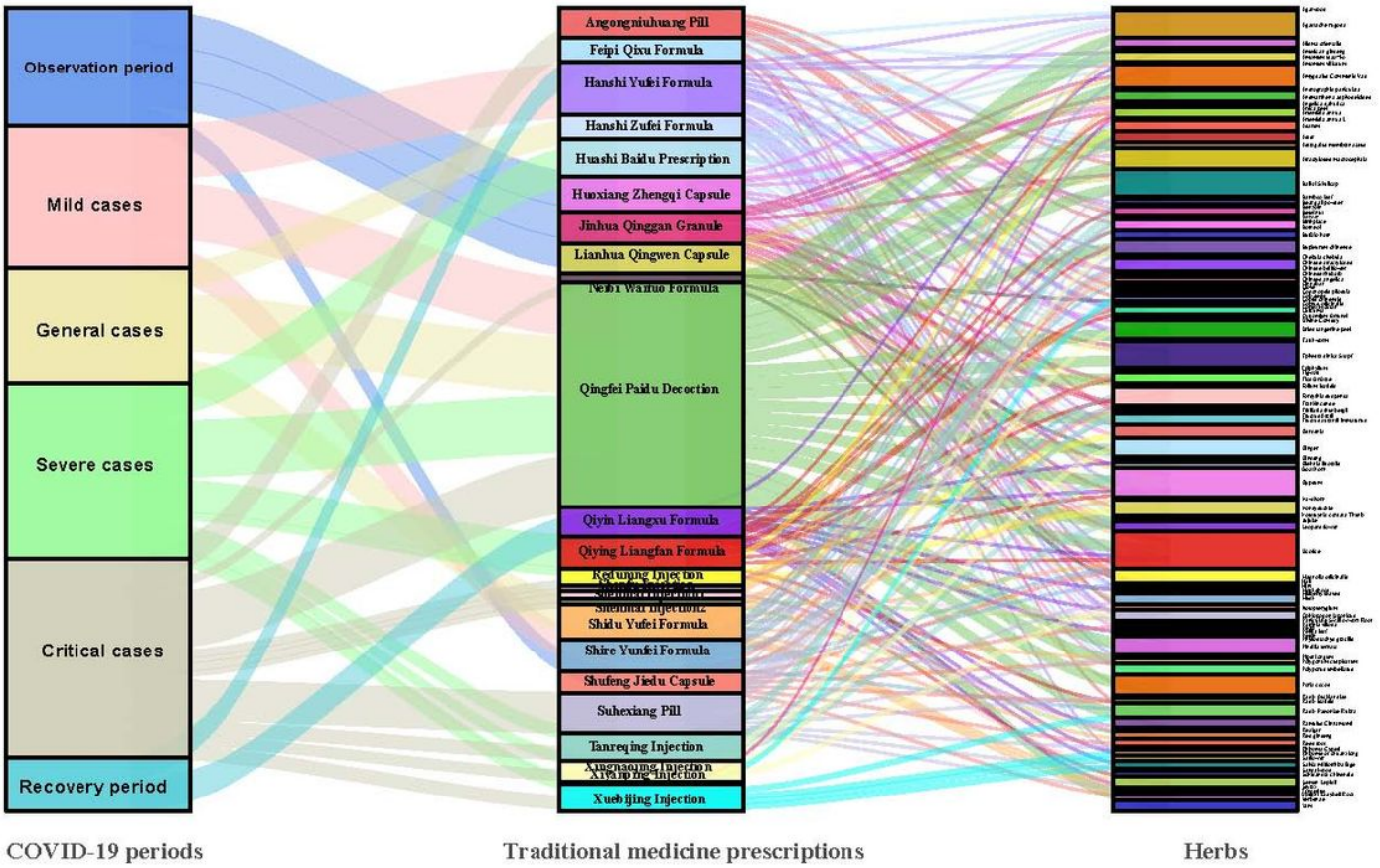

Figure 1

Distribution of traditional medicine for the treatment of COVID-19 in different stages

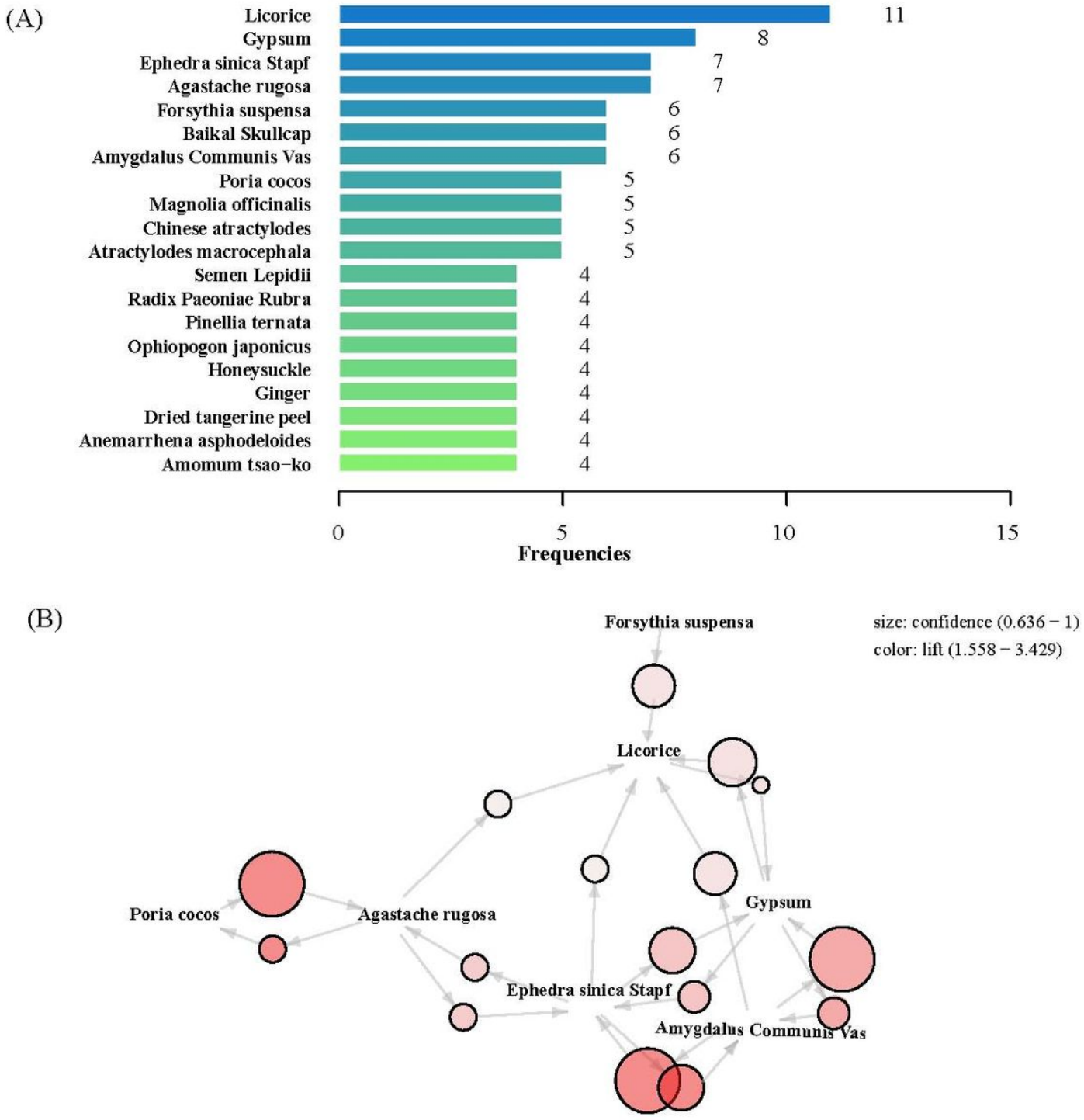

Figure 2

Distribution rules of herbs in the 24 recommended prescriptions. (A) The top 20 most commonly used traditional herbs in the 24 recommended prescriptions. (B) A graph for 16 rules with only two herbs. The larger the circle between the two herbs, the higher the confidence level, and the darker the color, the higher the lift value.

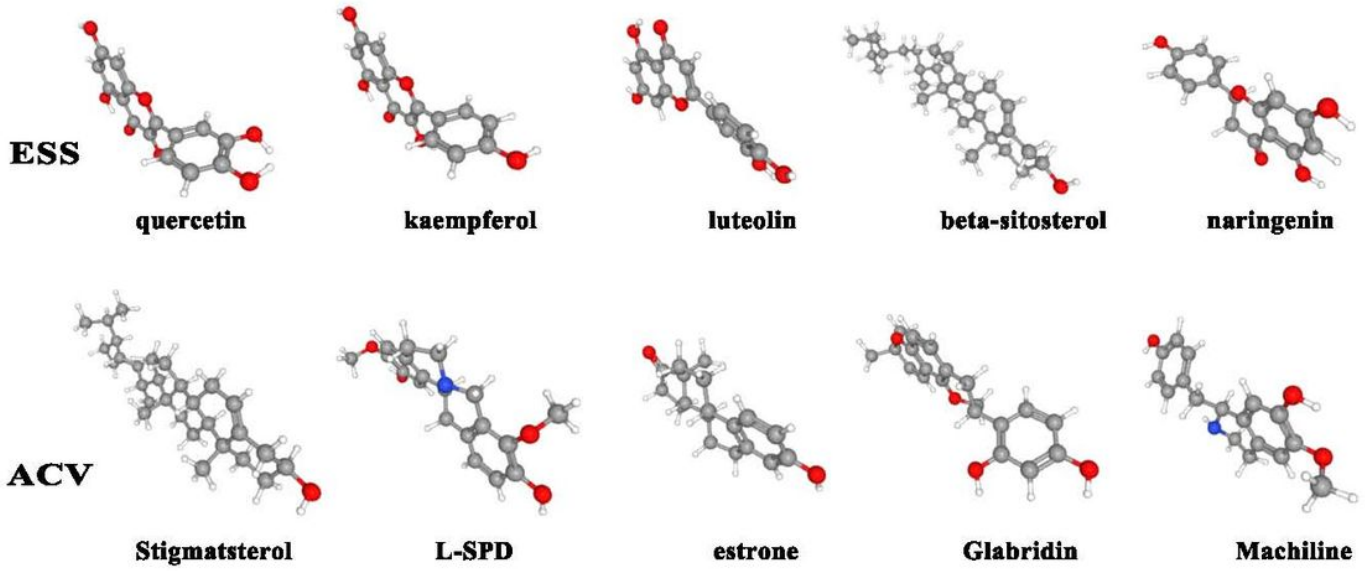

Figure 3

The top five compounds with the largest number of targets in ESS and ACV.

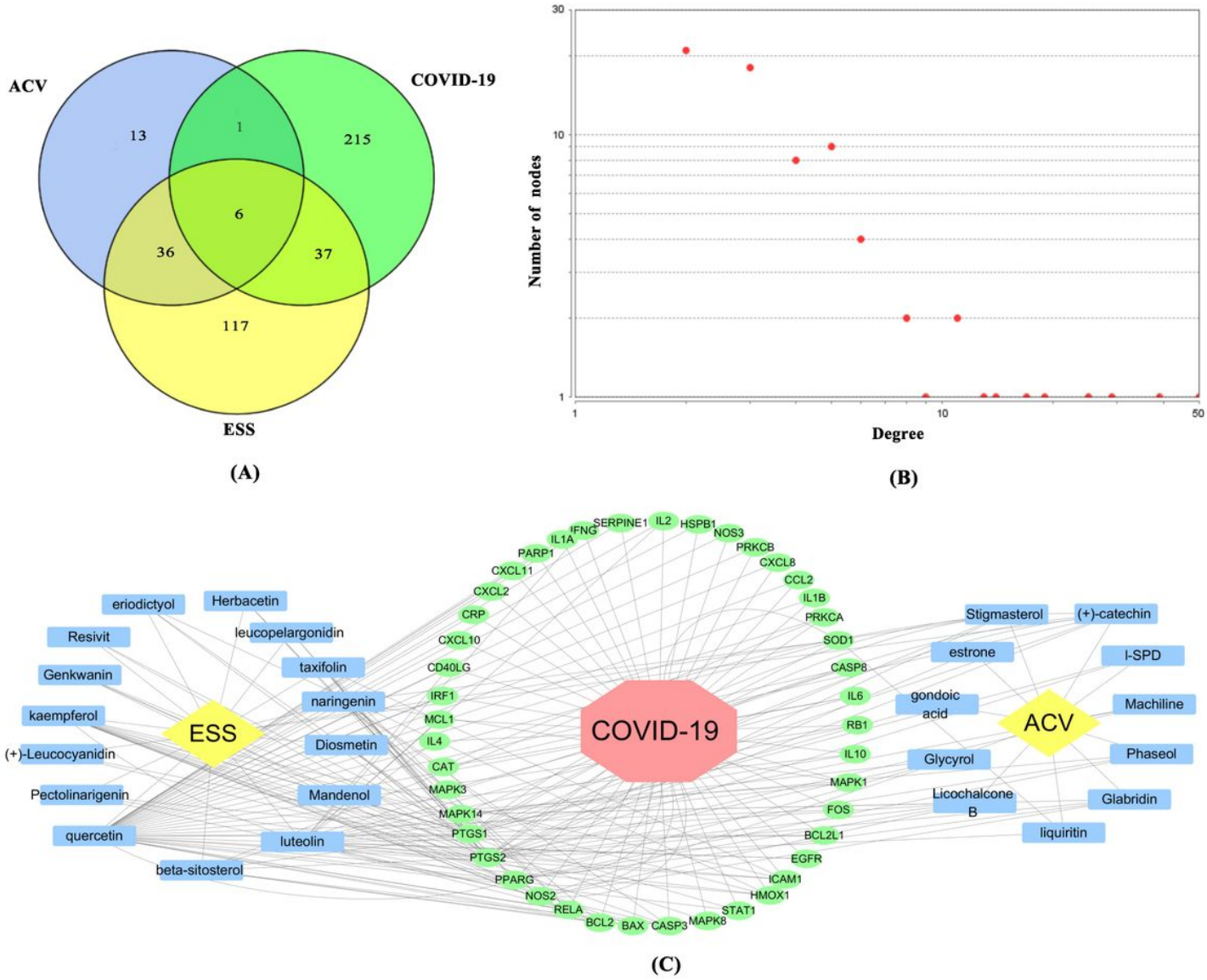

Figure 4

The “AE-component-target-COVID” network. (A) The crossed targets of ESS, ACV and COVID-19. (B) The node-degree distribution of “AE-component-target-COVID” network. (C) The “herb-component-target-disease” network of AE against COVID-19.

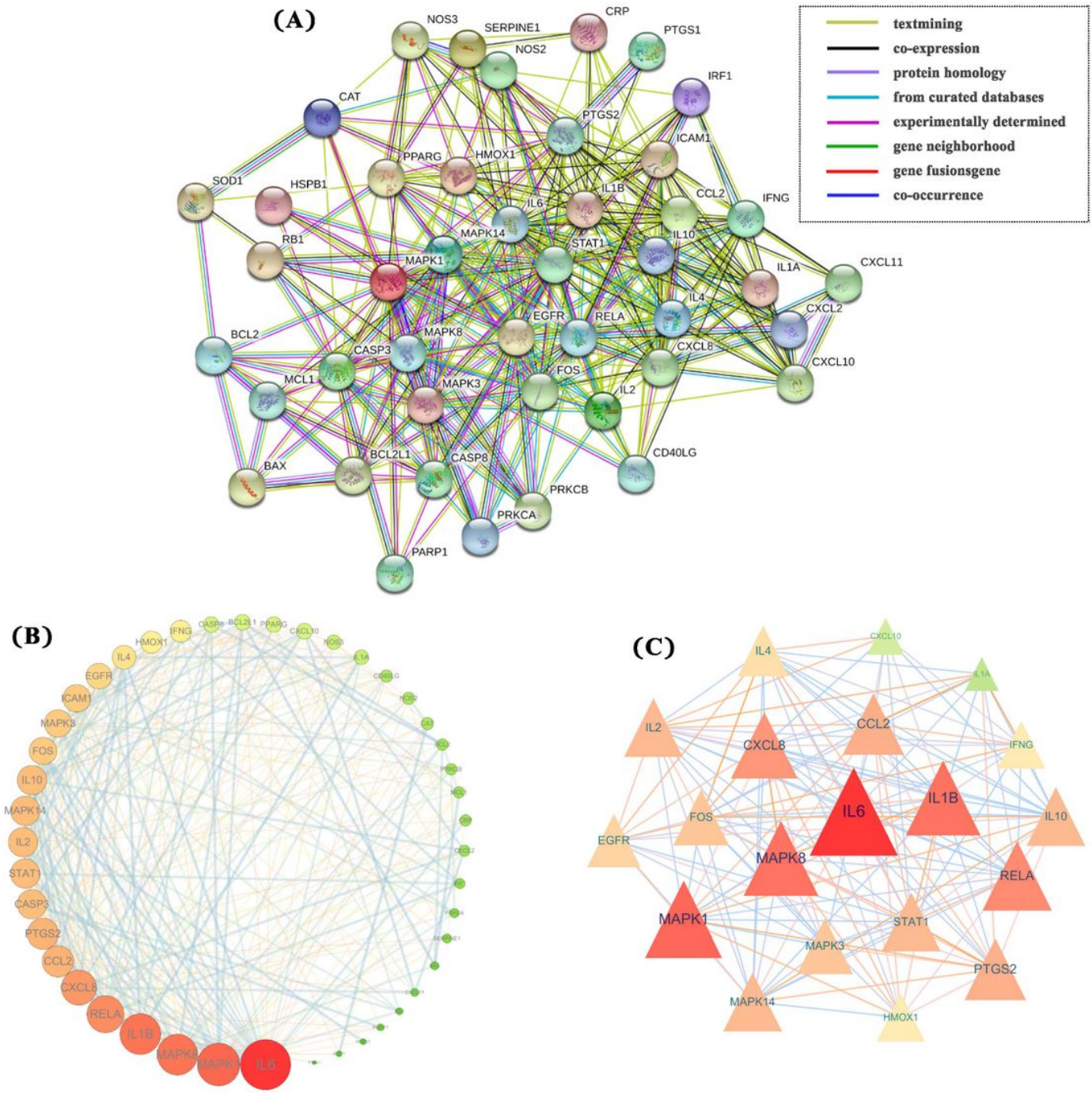

Figure 5

PPI network of AE compound targets againstCOVID-19. (A) The original PPI data generated from the STRING database showing the detailed interactions of the targets. (B) The PPI network constructed using Cytoscape, where the redder and bigger nodes represent vital targets. (C) The main cluster generated from the most important target, IL-6.

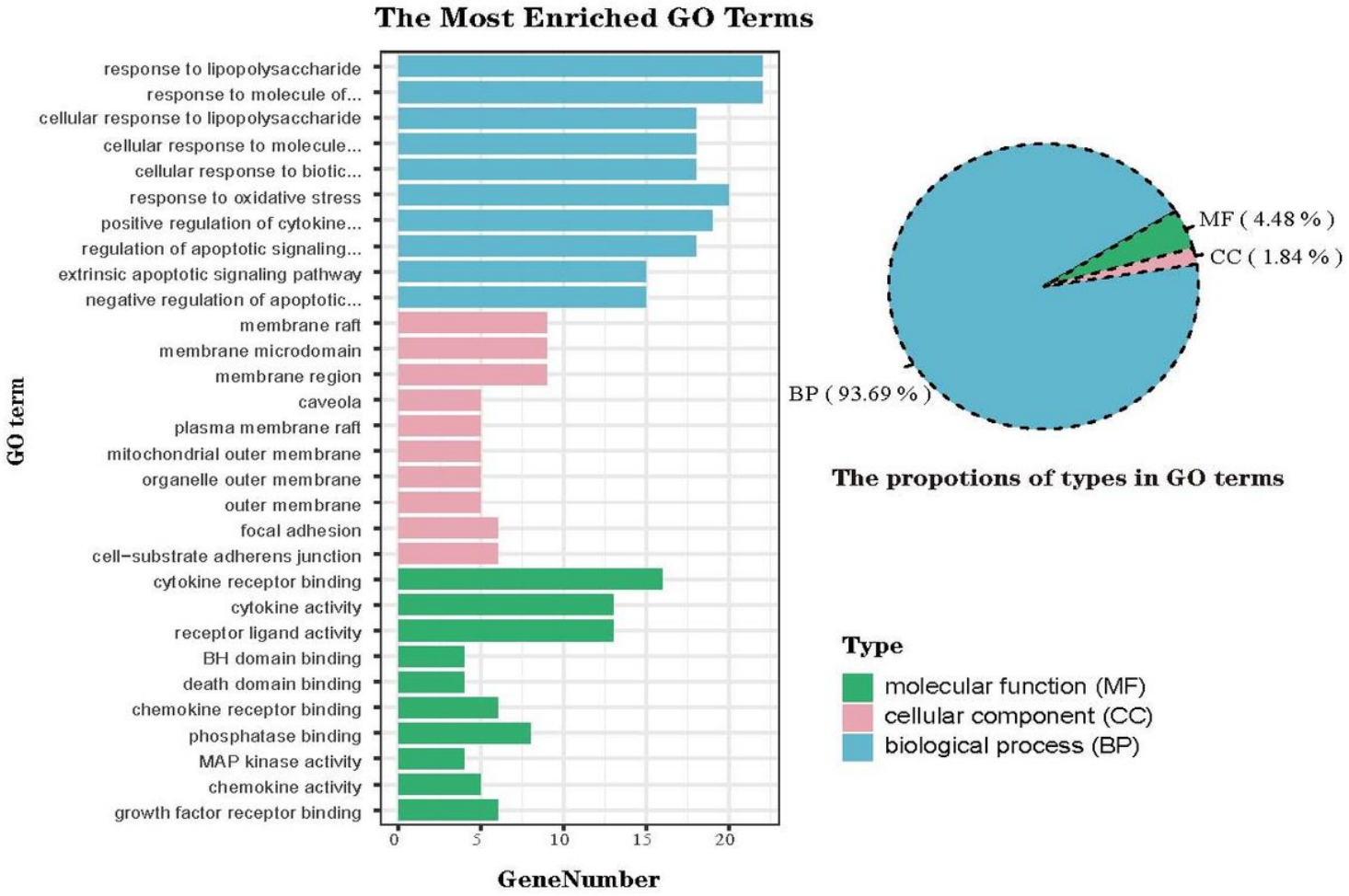

Figure 6

GO enrichment for the potential targets of AE. A list of enriched GO terms in relation to the potential targets of AE. In MF, CC, BP, the top 10 significant items were displayed respectively.

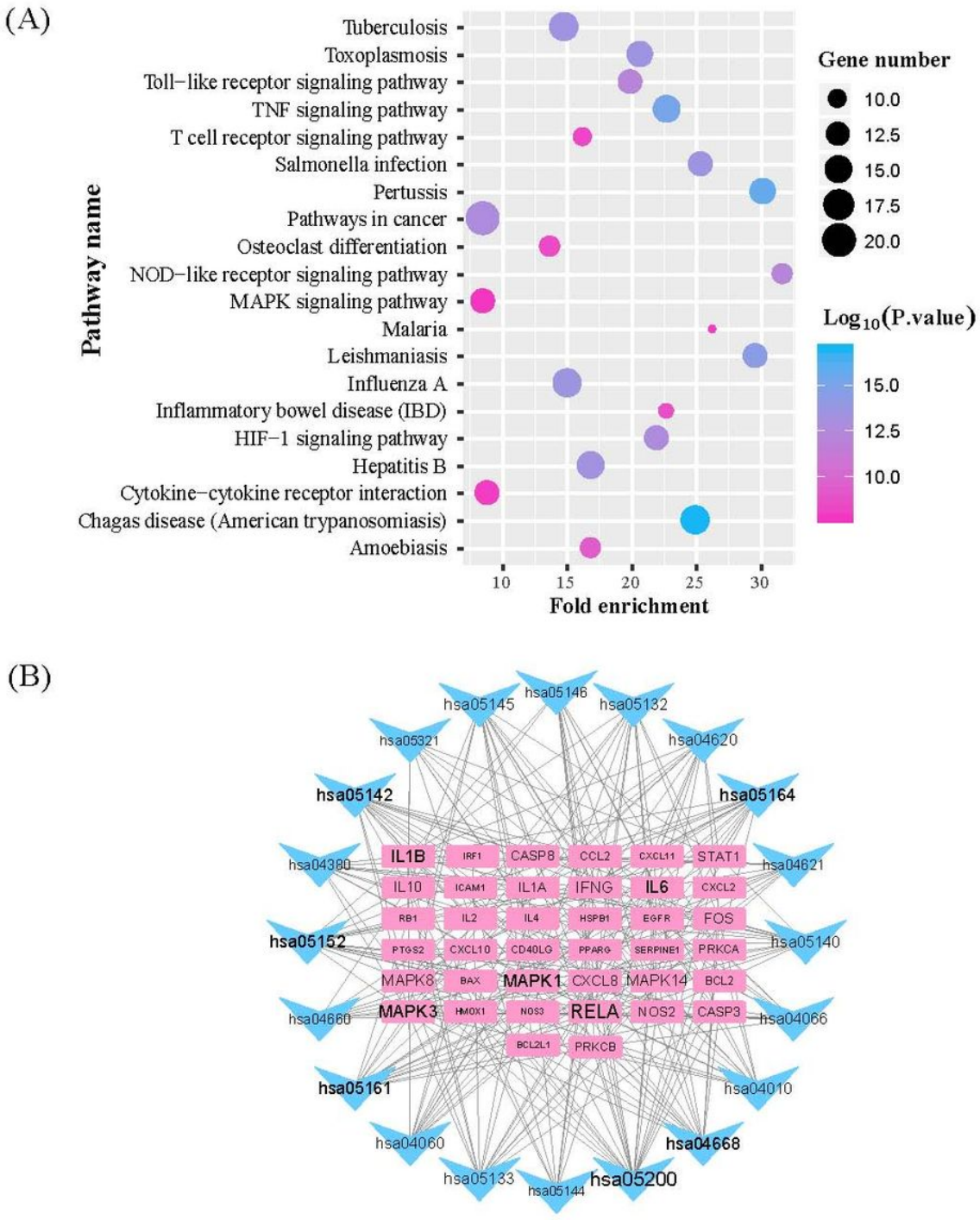

Figure 7

The Top 20 significant KEGG pathway analysis of shared targets for AE and COVID-19. (A) The KEGG pathway enrichment. The X-axis showed the enrichment scores of the pathways, while the Y-axis showed enriched GO categories of the targets. The size of the dots represented the counts of the genes, and the colour of the dots represented the P-value. (B) The Top 20 significant pathways and target networks. Blue represents the pathway ID and red represents the potential target. The larger the font, the greater the degree value of the node in the network.